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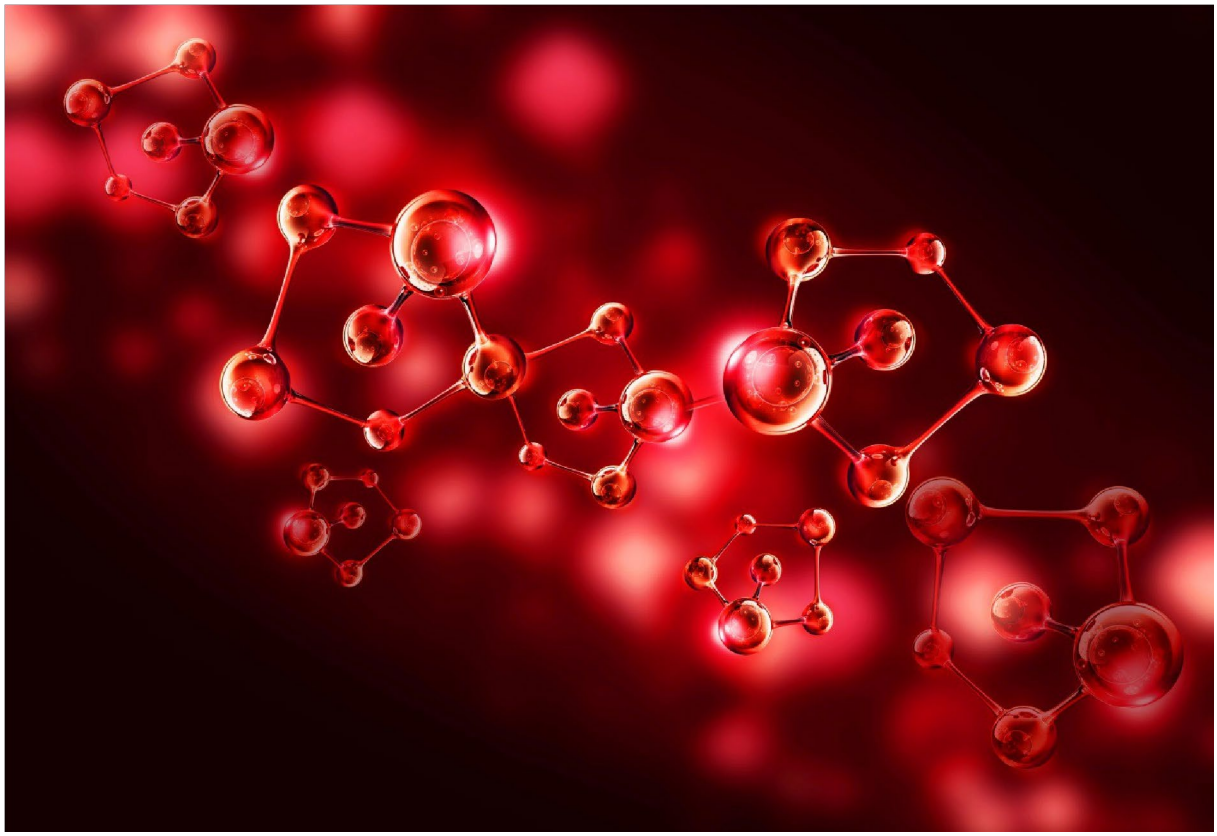

ABSTRACT BOOK 2022

Bachelor's and Master's Theses in Biomedical Laboratory Science

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Implementation and method verification of LC-MS/MS analysis of buprenorphine, fentanyl and norfentanyl in urine

By Alec Abarca Nilsson

Bachelors thesis in Biomedical Laboratory Science performed at the Department of Clinical Chemistry, Norra Älvsborgs Länssjukhus, 2022

Supervisor: Johanna Rudbäck (biomedical chemist), Elmira Luks (biomedical chemist)

BACKGROUND: Buprenorphine and fentanyl are both strong opioids with useful purpose in opioid use disorder and pain killing respectively, however both suffer from an addictive potential. To show proof of consumption liquid chromatography coupled to mass spectrometry (LC-MS/MS) analysis is performed in urine to quantify these substances along with the fentanyl metabolite norfentanyl.

AIM: The purpose of this study was to implement a validated LC-MS/MS method for quantitation of buprenorphine, fentanyl and norfentanyl in urine on a backup instrument and verify that it meets the standards of acceptable performance as well as showing comparable quantitation to current instrument in use.

METHOD: Settings on the collision cell and ion source in the mass spectrometer were optimized. Within-day (N = 10) and between-day (N = 5 per day, 5 days) imprecision and accuracy was tested with low and high control samples. Patient samples (N = 20 for each analyte) were analyzed, first on the current instrument, and then on the new instrument. Lower limit of quantitation was achieved by analyzing a serial dilution of the low control sample. Matrix effect was evaluated by post-column infusion of the analytes on blank samples and drug-free urine samples. Lastly, Carry Over was tested by analyzing the lowest calibration sample followed by the highest and then blank samples (N = 3) in that order.

RESULTS: Collision cell optimization was successfully done. However, ion source optimization failed, hence settings from the current instrument was implemented on the backup. The instrument showed excellent precision and accuracy both within-day ($CV \leq 4{,}07\%$, 97,5 – 109% accuracy) and between-day ($CV_{RW} \leq 5{,}65\%$, 101 – 107% accuracy). The backup LC-MS/MS displayed near perfect linear correlation ($r^2 \geq 0{,}998$) to the current LC-MS/MS for all analytes and insignificant bias in quantitation with Bland-Altman analysis. The lower limit of quantitation of buprenorphine, fentanyl and norfentanyl was calculated to be 1,25; 1,00 and 1,45 µg/L respectively. Significant ion suppression was observed for norfentanyl, but this will be compensated for by the internal standard. Carry Over was measured to be at most 6,32% for the first blank sample and 0,9% for the second and third samples.

CONCLUSION: The backup instrument has proven to perform the LC-MS/MS analysis of buprenorphine, fentanyl and norfentanyl in urine in accordance to predetermined criteria and can be justifiably used for drug testing of clinical patient samples.

Concordance between a monoexponential and a biexponential calculation of absorbed kidney doses in patients who have undergone their first ^{177}Lu-DOTATATE treatment

By Nesrin Abd El-Rahman

Bachelor thesis in Biomedical Laboratory Science performed at the department of clinical physiology at Östra hospital, Sahlgrenska Academy, University of Gothenburg 2022.
Supervisor: Jehangir Khan, PhD.

Background
Neuroendocrine tumors can be treated effectively with peptide receptor radionuclide therapy such as ^{177}Lu-DOTATATE. In radionuclide therapy, the kidneys are risk organs, so it is essential to monitor for the absorbed doses in the kidneys during therapy. This can be done using kidney dosimetry based on activity curves, which are formed by exponential functions (curve fitting models). The two most current functions are *monoexponential*, which is the golden standard, and *biexponential*, which provides an opportunity for observation of absorbed doses from uptake to elimination. Some studies argue that a monoexponential function provides more truthful values than a biexponential function, while other current studies reason that biexponential functions are equally suitable to use.

The aim of this study was to investigate for the concordance between a monoexponential calculation and biexponential calculation of absorbed doses in patients who have undergone their first ^{177}Lu-DOTATATE treatment and whether the latter in such cases could be used instead of the former in the clinic.

Method
The study included 17 patients who all underwent their first ^{177}Lu-DOTATATE treatment between 2019-2020. For each patient, 4 SPECT / CT scans were collected over the abdominal region 4, 24, 48 and 168 hours after infusion. Thereafter, the right and left kidneys of all patients were manually segmented based on the CT and SPECT images by applying volume of interest, and the activity concentration was obtained. Based on the activity concentration, the absorbed dose was calculated for all patients with both monoexponential and biexponential function.

Results
The median of the monoexponential calculation in the right side were 3.65 Gy (range 2.2-5.4 Gy) and 3.6 Gy in the left (range 2.4-5.4 Gy), and the median of the biexponential calculation in the right side were 4.12 Gy (range 2.4-6.5 Gy) and 3.79 Gy in the left (range 2.3-5.6 Gy). Scatterplots indicated and illustrated for a strong relationship between the two functions with R-value 0.89 in the right side and 0.87 in the left. A statistically significant difference between the models was obtained with a p-value of 0.01 in the right and 0.008 in the left side (Wilcoxon).

Conclusion
In conclusion, it is possible to use both models to measure absorbed kidney doses and obtain concordant results. However, it is appropriate to use a monoexponential function when imaging begins after 4 hours, and a biexponential function when imaging begins after 3 hours, or earlier.

Development of molecular cytogenetic method for risk stratification of multiple myeloma, validation of a method

By Khadijorahmo Abdullahi

Bachelor thesis in Biomedical Laboratory Science performed at the Cytogenetic laboratory at Sahlgrenska Academy, University of Gothenburg, 2022
Supervisor Helene Sjögren, Cytogeneticist, Med. dr., Dental surgeon

Background: Multiple myeloma is a chronic malign hematologic neoplasm that causes clonal proliferation of plasma cells in the bone marrow. The disease is characterized by a number of high-risk biomarkers, including t(4;14)(p16;q32), t(14;16)(q32;q23), deletion of TP53, 1q21 gain and t(11;14)(q13;q32) translocation. These biomarkers determine the patient's prognosis and preferred treatment. Fluorscence in situ hybridization is a molecular biology technique that enables the analyst to localize these biomarkers using different locus specific probes that bind to the DNA. The analysis is based on CD138 positive cells that are analyzed used different probes to localize chromosome aberrations.

The aim of this project is to increase the efficiency of the analysis that today takes too long in relation to the timespan set by the Swedish patient care bundles, which is 21 days. This is mainly because of the dropped slides with the cells being in the freezer for at least seven days. The timespan includes the time from the physicians suspect the disease to the time treatments is started. Simultaneously, the aim is to increase the quality of signals to get a more correct diagnosis. In this project two different preparation methods are compared to determine if they give the same results and to determine which one has the better signal quality. The preparation methods are the conventional method, ie manually dropping of cells on slides and the compared method is putting the cells on the slide by using cytospin.

Results: Both methods gave a comparable percentage of chromosomal aberrations. However, the cells that were dropped manually had slightly better quality. The timespan of the analysis was shortened by removing "aging" the cells in freezer for a week. In this study, we conclude that the cells show the same quality with or without this step.

Conclusion: In conclusion, this project has shortened the time of analysis of myeloma. However, the quality has not been improved. Hence, the current method with some modifications will be used in the future. The modification will be to eliminate aging of the cells in the freezer. In the future, new trials will take place to improve the quality of cytospin cells as this method has advantages both when handling the tests and for the environment, due to less plastic material being used.

VERIFICATION OF AN LC-MS/MS METHOD FOR THE QUANTIFICATION OF METHYLMALONIC ACID IN SERUM

By: Marwa Abdulmouti

Bachelor thesis in Biomedical Laboratory Science performed at Clinical Chemistry, Area 4, Norra Älvsborgs Länssjukhus in Trollhättan, 2022

Supervisors: Johanna Rudbäck, Elmira Luks.

Methylmalonic acid (MMA) is a methyl derivative of malonic acid and participates in the metabolism of fats and proteins in the body. MMA is a biomarker of intracellular vitamin B12 (B12) deficiency, so increased levels of MMA may indicate a B12 deficiency. Thus, MMA is counted as a complement to B12 analysis when investigating B12 deficiency. B12 participates in the body as a co-enzyme in two enzyme reactions that take place in mitochondria and the cytoplasm, respectively. B12 is necessary for the body's metabolism and cell function and is required for the central nervous system's function and development.

The project aimed to verify a method for liquid chromatography in combination with mass spectrometry (LC-MS / MS) to be used in the investigation of B12 deficiency by analyzing MMA levels in serum using an Agilent 1290 Infinity II liquid chromatography (LC) system connected to an Agilent 6470 triple quadrupole mass spectrometer (MS / MS) to a new LC-MS / MS instrument similar to the old instrument and to be used as a backup instrument. The work has a great importance as an additional instrument can be used to run tests if one of those instruments is out of order, so patient samples are not delayed.

The work included various tests used in verification of an LC-MS / MS method. The tests were performed in order to study precision, accuracy and method comparison by analyzing serum samples from 20 patients on both instruments. The verification started with optimization of the analyte fragmentation, where optimal parameters such as the fragmentor voltages and collision energies were determined on the new instrument. Verification tests were then performed.

The results of the study were approved for intermediate precision for low control, as well as intra-series precision according to the scope of accreditation for MMA, which has a limit of precision and accuracy of 7% -10% difference. However, the intermediate precision for high control was not approved according to the accreditation scope limit for MMA. At the same time, the difference for several test results was more than 15%-20% between the instruments in the method comparison. The ion suppression tests performed in order to study potential matrix effects, showed signal enhancement. However, this is acceptable when using isotope-labeled internal standards that compensate for the matrix effect.

The conclusion of the study is that the LC-MS / MS method in the new instrument is not allowed to be used in the analysis of MMA in patient samples.

Verification of mutation analysis for *KIT* D816V with digital PCR for implementation in clinical use

By Ally Abrahamsson

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Genetics and Genomics, Sahlgrenska University Hospital, 2022

Supervisor: Anna Staffas, PhD, Assistant Clinical Laboratory Geneticist and Assistant Supervisor Ulrika Odén, Biomedical Scientist.

The project's purpose was to test a multiplex method which detects a specific mutation in the *KIT*-gene, c.2445A>T and corresponding amino acid substitution p.Asp816Val. The mutation can be found in patients with systemic mastocytosis. The detection of this mutation is a diagnostic criterion for patients with systemic mastocytosis. In some patients it can be hard to find the mutation because of low variant allele frequency, which means the proportion of mutated copies in the DNA is low. And because of the low variant allele frequency the detection method must be a highly sensitive method. The method use in this project was droplet digital PCR multiplex assay from Bio-Rad laboratories. In the method a sample is partitioned to approximately 20,000 droplets which in every droplet a reaction occurs. After the PCR-reaction a reading of the droplets takes place to determine which droplets contains mutated copies. This assay uses TaqMan probes and the fluorescent colors FAM/HEX.

The project aimed to optimize and verify droplet digital PCR multiplex assay so that it could be used in clinical routine, because the current singleplex droplet digital PCR has issue with the interpretation of results. The singleplex uses a hydrolyses probe labeled with a fluorocom and a mutation-specific antisense primer. The optimization step in the project included testing if the addition of restriction enzyme HaeIII was beneficial and investigation of the optimal DNA input. HaeIII turned out to be not useful for this assay and input of 40-200ng DNA showed to be the most efficient DNA input. In the verification of the method five different parameters was examined, precision, limit of detection, method comparison, accuracy, and linearity testing. In the precision step a run controls were analyzed in triplicates in four rounds, and it resulted in a coefficient of variation 7,41%. Background noise from 18 samples were used for limit of detection and they were assumed to be negative for *KIT* D816V. The result led to a limit of detection of 4 positive droplets corresponding to a variant allele frequency of roughly 0,017-0,025%. In the method comparison 10 positives and 10 negative samples of *KIT* D816V was used, and the result showed a good conformity with the current method. The accuracy was determined by analyzing 10 external control samples and it proved to be a good correlation, with an R^2 value of 0,98 between the observed result and the accurate result, that were based on the consensus values reported from other clinical laboratories. The linearity testing showed that the assay has a good quantification ability down to the limit of detection. The overall conclusion was that the droplet digital PCR multiplex assay meets the requirements of implement the method for clinical use.

CAN NO EFFECT BE SEEN IN INTESTINAL INFLAMMATION RELATED TO DIETARY FIBERS IN WOMEN AFTER PELVIC RADIOTHERAPY?
- A longitudinal study of cytokines and inflammatory markers in blood.

By Ellen Athley

Bachelor thesis in Biomedical Laboratory Science performed at the University of Gothenburg
Sahlgrenska Acadamy, University of Gothenburg, 2022
Supervisor: Fei Sjöberg, reader

Background: Women who receive radiotherapy for gynecological cancer often get persistent and far-reaching negative symptoms from the intestine. Radiotherapy damages the intestine and prolonged inflammation can cause negative bowel symptoms. Dietary fiber could be used to reduce intestinal inflammation after radiation treatment and furthermore lower negative bowel symptoms. However, the evidence is weak and the dietary advice at oncology clinics in Sweden varies. **Aim**: To investigate the effect of dietary fiber on radiation-induced inflammation of the gastrointestinal tract in women who have undergone radiotherapy for gynecological cancer. **Method**: Participated were 16 women who had received pelvic radiotherapy. The women received psyllium as a dietary supplement and were divided into two groups, adequate fiber intake versus insufficient fiber intake. Inflammatory cytokines and CRP were analyzed in a total of 64 serum samples from four timepoints by using Proximity Extension Assay and Enzyme-linked immunosorbent assay respectively. The occasions ranged from before the radiation treatment to one year after the treatment had ended. The difference between the groups where analyzed. **Results**: No significant difference could be obtained between the groups regarding any cytokine or CRP at any one time. A spread among individuals in both groups is found on all four occasions. **Conclusions**: No coherent cytokine profile could be seen, and no significant difference could be established in this study. Another study design without differential comparison, with more participants or other dietary supplements, might have led to other results. This study cannot influence any recommendation of dietary supplements to women who have received pelvic radiotherapy. Further studies are recommended.

Toll-like receptor 3 signaling in human heart fibroblasts downregulates the gene expression of gamma-protocadherin's.

By: Camila Axelsson

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Chemistry, Sahlgrenska University Hospital, University of Gothenburg 2022.

Supervisor: Victoria Rotter Sopasakis PhD & Lillemor Mattsson Hultén Professor.

Introduction: Chronic inflammation in the human heart often occurs due to heart disease and leads to damages. The heart has three major cell types, cardiomyocytes, fibroblasts, and vascular cells. The role of cardiac fibroblasts regarding chronic inflammation in the heart has not yet been established. The aim of this project was to investigate whether there are unique genes in the NF-κB signaling pathway that are regulated by toll-like receptors 1,3 and 7 in cardiac fibroblasts. In addition, identify whether there is a connection between toll-like receptor signaling and gamma protocadherin's as well as microRNA's.

Method: Fibroblasts from healthy individuals were incubated with three toll-like receptor agonists, PamCSK3 induces toll-like receptor 1 signaling, poly I: C induces toll-like receptor 3 signaling and R848 induces toll-like receptor 7 signaling. A SYBR-Green based qPCR array with 84 unique genes for NF-κB-signaling was tested with all agonists. The selected genes, including protocadherin's and microRNAs, were also tested with the agonists but were analyzed with Taqman qPCR.

Results: The results showed that all agonists activated the NF-κB signaling in the cardiac fibroblasts. The agonist poly I:C activated19 unique genes in the NF-κB signaling pathway compared to PamCSK3 and R848 which only activated two and five unique genes respectively. Genes that were previously shown to be downregulated in human heart tissue including gamma-protocadherin's, were also downregulated in the cardiac fibroblasts. The agonist poly I:C showed a significant downregulation in eight protocadherin's while the other two agonists showed a significant downregulation in only one gamma protocadherin gene. The toll-like receptors did not affect the expression of the microRNA's.

Conclusion: In summary the cardiac fibroblasts are a strongly contributing cell type for inflammation in heart tissue and signaling via toll-like receptor 3 seems to activate most genes in the NF-κB signaling pathway and downregulate gamma protocadherin's.

Ionized calcium in venous serum can be reused for analysis up to 240min after initial analysis

By: Bang Thanh Tran

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Chemistry, Sahlgrenska University Hospital, University of Gothenburg, 2022.

Supervisor: Ruth Wickelgren, PhD.

Introduction: Ionized calcium is the clinical parlance of free calcium ions in the extracellular matrix and is critical for maintaining calcium homeostasis, in which imbalances is associated with calcium metabolic disorders.

In vivo, the free cation is in an equilibrium with albumin bound calcium which is sensitive to pH. The sensitivity causes preanalytical errors that entails difficulties. One source of error is that the seal of evacuated serum tubes must be removed before analysis. Upon the removal the specimen is exposed to oxygen and suffers a loss of carbon dioxide which causes pH changes that alters the ion concentration. The aim of this project is to examine if the same results could be obtained between an initial analysis of ionized calcium in venous serum and a rerun.

Method: The study consists of three test groups; filled tubes, half-filled tubes, and tubes with pathological ion concentration. For the first two groups the seal was removed and resealed in 15 seconds to imitate a used tube. For all groups ionized calcium was determined with potentiometri by a blood gas instrument. Filled tubes were analyzed at initial time 0min and reanalyzed at 30, 60, 120 and 240min respectively. The half-filled tubes and tubes with pathological ion concentration were analyzed at 0 and 240min.

Results: The main finding was that ionized calcium analyzed in filled, half-filled tubes and tubes of pathological ion concentrations at initial time and given times show no statistical significance between the measurements. A positive correlation and concordance within the limits of agreement was shown for every test group and each group did not exceed the limit for coefficient of variation of 2%.

Conclusion: In conclusion, ionized calcium in serum can be reused for analysis up to 240min after initial analysis. Given that the seal is opened and closed within 15 seconds.

SPECIES DETERMINATION OF BAUMANN'S COLLECTION OF MARINE BACTERIA WITH 16S rRNA SEQUENCING

By Nikolai Basic
Bachelor thesis in Biomedical Laboratory Science performed at Clinical Microbiology, Sahlgrenska University hospital, Gothenburg, 2022
Supervisor: Liselott Svensson Stadler, PhD

In 1971 a Baumann study of 218 marine bacteria were screened with extensive biochemical and morphological and tests for species determination. Based on the results they were divided into 21 groups. In this study we aim to taxonomically classify the Baumann collection using 16S rRNA sequencing. Some of the isolates were expected to not be correctly determined due to previous methods having lower resolution as well as a smaller library of known bacterial species for comparison. We expect to find strains where the species identification is no longer accurate as well as not previously published species. The 16S rRNA gene was amplified with PCR from bacterial DNA. Capillary Sanger sequencing was performed and the results were analyzed in BioNumerics software. Type strain sequences for comparison were collected from Genbank (NCBI). With less than 1,4% difference in the gene between two sequences it was assumed that they were the same species.

83 strains of the 218 were sequenced. Six of these were novel genus or species, 30 were undetermined species due to high similarity to multiple species and 46 were classified with genus and species. The results were divided based on Baumanns groups as follows. Group E1 was identified as *Alteromonas* sp. All A1 were *Marinomonas communis*. C3 was now *Ocenanimonas duodorffii*. H1 was *Oceanobacter kriegii* and A2 was *Oceanospirillum vagum*. B2 was identified as *Phaeobacter italicus* and C1 as *Leisingera sp*. H2 was identified as *Halarcobacter novel sp*. B1 was identified as novel genus. C2 was now *H. pacifica*. In group D2 one isolate was *H. cupida*, one was *H.organivorans* and one was *Halomonas sp*. D3-D4 and F1 were identified as two different *Halomonas sp*. In group E2, three isolates were *Pseudoalteromonas sp*, one isolate identified as *Pseudoalteromonas mariniglutinosa*, and one isolate as another *Pseudoalteromonas.* F2 was *Cobetia sp*. In group G1 seven isolates were *Marinobacterium stanieri*, one isolate was *M. novel sp* and one isolate was *M. georgiense*. D1 was *M. novel sp*. In group G3, nine isolates were *Marinobacer nauticus*, two isolates were *M. daepoensis* and *M. xestospongiae*, respectively

In conclusion a large part of the collection was taxonomically classified with 16S rRNA sequencing. Some species had been previously classified as inaccurate species or genus in addition to many name changes that have taken place over the years. The six isolates that are new (less than 98,6% similar to any described species) will be sequenced and analyzed using Digital DNA-DNA hybridization and Average Nucleotide Identity and described as new species. The 30 strains that could not be determined (more than 98,6% similar to multiple species) will be targeted with housekeeping genes.

SURVIVIN-DEPENDENT CD4$^+$ T-CELLS DURING JOINT INFLAMMATION

by Robert Breiðfjörð

Bachelor thesis in Biomedical Laboratory Science performed at
The Department of Rheumatology and Inflammation Research, Sahlgrenska academy, University of Gothenburg, 2022
Supervisors: Maria Bokarewa, Professor/Senior rheumatologist
Eric Malmhäll-Bah, PhD student

Background: Cellular processes behind the development of join inflammation in rheumatoid arthritis is not fully understood, macrophages and CD4$^+$ T-cells accumulate in the synovium and together they maintain the inflammation in the joints. To better understand the behaviour of CD4$^+$ T cells during joint inflammation conditional knockout mice that lack functional GGTase-I in their macrophages (GLC-mice) are used. GGTase-I deficiency leads to a hyperactivation of macrophages and subsequent joint inflammation. Previous experiments have shown an accumulation of activated CD4$^+$ T-cells in lymph nodes and spleen of GLC-mice with increased tendency to migrate and invade tissue. Inhibition of survivin-expression has previously been shown to relieve joint inflammation.

The project aimed to inhibit the expression of survivin in GLC mice and examine the effects on CD4$^+$ T-cell behaviour and the mobilisation of regulatory T-cells from thymus during joint inflammation.

Method: In two independent experiments GLC-mice were treated with YM155 (n = 16) or lentivirus particles with shSURV (n= 20) to inhibit survivin. Relative gene expression in CD4$^+$ cells from the lymph nodes and spleen were analysed with two-step RT-qPCR for Cdc42, Rac1, Rhoa, Pggt1b, Foxp3, Ikzf2, Lef1, Ctnnb1, Lrrc32, Itgb1, Nrp1, Vim, Hoxa10, Birc5

Results: YM155-treated mice had a lower relative gene expression of markers regarding cell migration and mobilization of regulatory T-cells with thymic origin in cells from lymph nodes but not the spleen. Mice treated with shSURV had no significant differences in relative gene expression for any markers compared with control mice.

Conclusion: The inhibition of survivin expression in GLC-mice influenced the migration of activated CD4$^+$ T-cells to lymph nodes. The results indicate that the accumulation of activated CD4$^+$ T-cells in lymph nodes and the mobilisation of regulatory T-cells of thymic origin to the lymph nodes during joint inflammation are survivin-dependent processes.

Effects of COVID-19 on arterial stiffness and vascular aging in young, healthy adults – A cross-sectional study

By Charlotta Carlsson

Bachelor thesis in Biomedical Laboratory Science performed at Coimbra Health School, Polytechnic Institute of Coimbra, 2022
Supervisor: Telmo Pereira, PhD

Introduction: The viral infection of COVID-19 has been shown to have a significant effect on the vascular system. However, there is yet to be clear evidence of an association between COVID-19 and arterial stiffness. The aim of this study was to assess the degree of arterial stiffness and presence of Early vascular aging (EVA) in subjects previously infected by SARS-CoV-2, in order to provide further understanding of the impact of COVID-19 on the vascular system.
Methods: A cross-sectional study was performed, investigating parameters of arterial function and structure in healthy, young adults. The sample size consisted of 40 participants, equally divided into two groups: a COVID-19 group in an early phase after infection of SARS-CoV-2 and a control group without previous infection of the virus. Methods used for evaluation were carotid-femoral pulse wave velocity (cfPWV) and carotid pulse wave analysis (PWA).
Results: Significantly higher values of PWV were found in the COVID-19 group compared to the control group. Similar results could be observed for several parameters obtained by PWA. These parameters included central systolic blood pressure (cSBP), brachial systolic blood pressure (bSBP), central pulse pressure (cPP) and brachial pulse pressure (bPP), all of which presented significantly higher values in the COVID-19 group. Additionally, a trend for higher values of augmentation index was found in the COVID-19 group.
Conclusion: This study indicates an increase of arterial stiffness and presence of EVA in young, healthy adults in the early phase after infection of COVID-19. Additionally, higher values of several haemodynamic parameters further conclude the effects on the vascular system.

Analysis of Intratumoral inflammatory infiltrate, Mismatch Repair Protein and PD-L1 expression in endometriosis associated ovarian cancer

By Huana Chen

Bachelor thesis in Biomedical Laboratory Science performed at the Clinical pathology, Sahlgrenska university hospital, Sahlgrenska Academy, University of Gothenburg, 2022

Supervisor: Claudia Mateoiu, PhD, doctor at gynecological pathology, SU, and Amanda Martinsson, Leg BMA.

Background: Endometroid (EOC) and clear cell ovarian cancer (CCC) are endometriosis associated epithelial ovarian cancer. These are also associated with Lynch syndrome (LS) which is caused by mutations in Mismatch repair genes leading to deficiency of the mismatch repair function (MMRD). MMRD has detected in 2% of patients with CCC and 18% of patients with EOC. Up to 10% of patients with LS may develop epithelial ovarian cancer. PD-L1 is a protein that suppresses the acquired immune system. Tumor cells use PD-L1 to inhibit the activity of T cells and to avoid being attacked by the immune system. With immunotherapy of anti-PD1 / PD-L1, the immune system can be restored against the cancer and used as a treatment for many different types of cancer. Previous studies have found that intratumoral stromal inflammation is associated with MMRD and PD-L1 expression.

The aim of this study is to evaluate the association between MMRD, PD-L1 and intratumoral stromal inflammation and correlation between endometriosis associated ovarian cancer and clinical pathological features.

Method: Selected patient cases with EOC and CCC were merged to a paraffin block with TMA method. Then stained with immunohistochemical staining to screen PD-L1 expression. The study uses data collected last year, which consists of the results from the expression analysis of mismatch repair proteins

Results: MMRD was found in 4 cases and PD-L1 expression in 20 cases. Of the four cases with MMRD, three of the cases showed positive intratumoral stromal inflammation, corresponding to 75%. For PD-L1, 90% showed positive intratumoral stromal inflammation.

Conclusion: No association was shown between MMRD and PD-L1 expression, but there has been a correlation between diffuse intratumoral stromal inflammation and MMRD. Correlation has also shown between diffuse intratumoral stromal inflammation and PD-L1 expression.

FLUORESCENS *IN SITU* HYBRIDIZATION FOR EXAMINATION OF BACTERIA INFILTRATION IN IRRADIATED CANCER SURVIVORS WITH CHRONIC INTESTINAL INFLAMMATION

By: Michaela Ekh

Bachelor thesis in Biomedical Laboratory Science, Sahlgrenska Academy, University of Gothenburg 2022

Preformed at the Department of Oncology, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg

Supervisors: Sravani Devarakonda, MSc Molecular Biology, Cecilia Bull, Docent

Background: Radiation-induced damage to intestinal tissue that has been in the field of radiation or in the vicinity is a problem for cancer survivors who have been treated with radiation to the pelvic area. The perception is that the acute inflammation with crypt loss and increased intestinal permeability quickly turns into fibrosis and ischemia, with a lack of continued inflammation. In contrast, a low-grade, chronic inflammation has been found in intestinal biopsies in cancer survivors.

Aim: By understanding the underlying causes of the chronic low-grade inflammation in the intestinal mucosa that the radiation causes, new opportunities are developed to develop preventive or even curative care. The aim was to investigate whether the chronic low-grade inflammation is caused by bacteria from the lumen penetrating through the intestinal barrier and into the mucosa.

Method: Fluorescence *in situ* hybridization is a method that allows visualization, identification, quantification and localization of microbes. With this technique combined with a modified method of stereology microscopy, bacteria were quantified in intestinal biopsies from cancer survivors who were irradiated against the pelvic area up to 20 years ago and suffer from long-term intestinal problems, and in unirradiated controls.

Results: An increase in bacterial infiltration was observed in both crypts and lamina propia in the biopsies taken from high-radiation areas, compared with healthy controls or low-radiation areas.

Conclusion: An underlying bacterial infiltration may be the cause of the low-grade inflammation seen in intestinal biopsies from irradiated cancer survivors. Future proof-of-concept studies may determine whether inflammation can be suppressed through strategies that prevent bacteria from crossing the intestinal barrier.

Developing macrophages culture in breast cancer patient-derived scaffolds (PDS) for the study of the immune modulation by the tumor microenvironment

By: Malin Engelin

Bachelor thesis in Biomedical Laboratory Science performed at Göran Landberg group, Sahlgrenska Center for Cancer Research, Sahlgrenska Academy, University of Gothenburg, 2022

Supervisor: Elena Garre: PhD, senior researcher

Co supervisor: André Holdfelt, PhD, associate researcher

Background: It has been shown that the tumor microenvironment (TME) plays a role in development and progression in breast cancer. Among the immune cells that are recruited to the tumor microenvironment, tumor associated macrophages (TAMs) are abundant in breast tumors. TAMs can adapt their phenotypes depending on the stimuli from the TME. Simplifying, they are generally divided into two types, the classical type (M1) and the alternative type (M2). The M1-macrophages have pro-inflammatory properties unlike the M2-like that are anti-inflammatory macrophages and promote tumor progression and metastasis and are associated with poor prognosis. To unravel the tumor microenvironment role in tumor progression, nowadays many studies are using *in vivo*-like 3D models to better represent the complex interaction between cells and the extracellular matrix *in vitro* than using the traditional 2D monolayer cultures. In this project patient-derived scaffolds (PDSs) from breast tumor tissue, developed in Göran Landberg group, are used. These decellularized scaffolds preserve the extracellular matrix and associated molecules, keeping relevant and unique information from the TME from each patient.
Aim: The aim of this study is to optimize the experimental conditions and find adequate readouts to eventually repopulate the PDSs with cancer cells and macrophages simultaneously and analyse the immune response modulation by the TME. To achieve this, it was analysed, firstly the capacity of the THP-1 monocyte cell line to repopulate the PDSs and respond to that microenvironment, and secondly, the capacity of the cancer cells to induce changes in THP-1 cells.
Methods: PDSs were repopulated with the THP-1 cell line and the cell infiltration was analysed in histological sections by H&E staining.. THP-1 co-cultures were performed with the cancer cell lines MCF7 or MDA-MB-231. In all the experiments, the changes in gene expression of M1-/M2- markers was analysed by qPCR to study the polarization of the macrophages.
Result: The THP-1 cells were able to infiltrate the PDSs and modify their gene expression. Co-cultures with MCF7 or MDA-MB-231 induced different responses in THP-1 cells, where the cells differentiated to M0-like macrophages showed more changes than THP-1 monocytes. Moreover, THP-1 cells growing with MDA-MB-231 showed induction of several M2-like markers, suggesting a polarization towards this state although more experiments need to be performed.
Conclusion: The differential changes in gene expression of the THP-1 monocyte cell line when growing in PDSs or in co-cultures with cancer cells suggest that it is a suitable cell line to monitor changes in the tumor microenvironment and study the immune response modulation using the PDS model.

Development of a LC-MS/MS method for separation of glucosylsphingosine and galactosylsphingosine to improve diagnostic possibilities for Krabbe Disease

By Erica Fransson

Bachelor thesis in Biomedical Laboratory Science at Clinical Chemistry laboratory at Sahlgrenska University Hospital, Sahlgrenska Academy, University of Gothenburg, 2022

Supervisor: Mette Diswall, PhD

Abstract

Background
At the laboratory of Clinical Chemistry at Sahlgrenska University Hospital there is no available analysis to measure the biomarker for Krabbe disease (KD) – galactosylsphingosine (GalSph). In one of the existing routine of analyses for lysosomal diseases, hexosylsphingosine (HexSph) is quantified as a biomarker för Gaucher Disease (GD). HexSph is a group of molecules that includes the specific biomarker for GD – glucosylsphingosine (GlcSph), and GalSph. These molecules are isobaric structural isomers with similar chemical characteristics, which makes them challenging to separate using liquid chromatography.

The aim of this project was to improve the diagnostic possibilities of KD by developing an LC-MS/MS method that is capable of separating GalSph from GlcSph. To ensure the diagnostic value of the method, the amount of respective analyte from control individuals and patients with GD and KD were compared.

Method
Two different LC-MS/MS methods were tested and their ability to separate GlcSph and GalSph was evaluated. One of them was based on a previously published study and the other one on a method in routine use at the laboratory. The chromatographic inlet was optimized to improve the separation of the analytes. EDTA-plasma and cerebrospinal fluid (CSF) from healthy individuals and patients diagnosed with either GD or KD were analyzed to examine the proportions of GlcSph and GalSph in the two diseases.

Results
The optimized chromatographic inlet was able to separate GlcSph and GalSph. The concentration of GalSph was considerably higher in Krabbe patients as compared to Gaucher patients, and the opposite was seen for GlcSph. In plasma and CSF from healthy individuals, neither analyte was elevated.

Conclusion
During this project an LC-MS/MS method with capability to separate GlcSph and GalSph was partly developed. After further development and validation, this analysis can be used as a second tier to determine if the elevated concentration of HexSph is caused by high levels of GlcSph or GalSph. This knowledge can be of diagnostic value when KD or GD is suspected.

Right ventricular dysfunction more prevalent in Takotsubo syndrome compared to anterior STEMI - an observational study with echocardiography

By Elin Gavelli

Bachelor thesis in Biomedical Laboratory Science performed at the department of Clinical Physiology, Sahlgrenska University Hospital, 2022
Supervisor: Sandeep Jha, Cardiology Consultant – Department of Cardiology (Sahlgrenska University Hospital), Emanuele Bobbio, Cardiology Consultant – Department of Cardiology (Sahlgrenska University Hospital), and Caroline Schmidt, Associate Professor.

Background and aim: Takotsubo syndrome is a condition first described in Japan in 1990. The clinical features and changes in electrocardiogram resemble those of acute anterior ST-elevation myocardial infarction. Although Takotsubo and anterior ST-elevation myocardial infarction primarily affect the left ventricle, right ventricular dysfunction could occur in some cases, which then correlates with increased complications, longer hospitalization and an impaired systolic left ventricular function. This study aims to examine the prevalence of right ventricular dysfunction in patients with Takotsubo compared to anterior ST-elevation myocardial infarction, using echocardiography.

Material and method: This study was an observational study within a larger prospective study; STAMI (Stunning in Takotsubo syndrome vs Acute Myocardial Infarction) at the Department of Cardiology, at Sahlgrenska University Hospital in Gothenburg. Echocardiography was performed at baseline (alternatively day 1 from admission) and day 7 ± 48 h. The final study population consist of 17 patients with Takotsubo and 44 patients with anterior ST-elevation myocardial infarction.

Results: The Takotsubo-patients indicated more often an involvement of right ventricular dysfunction at baseline, including the longitudinal function observed with TAPSE, compared to those with anterior ST-elevation myocardial infarction. Moreover, further analyses showed that the right ventricular function recovered on day 7.

Conclusion: The results of our study show that right ventricular dysfunction is more prevalent in patients with Takotsubo syndrome compared to those with anterior ST-elevation myocardial infarction, which is in agreement with the results of an earlier study.

Perfusion of whole ewe ovary- new fertility preservation method for young cancer patients

By Eliza Grusiecka
Bachelor thesis in Biomedical Laboratory Science performed at Laboratory for Transplantation and Regenerative Medicine, Sahlgrenska Academy, University of Gothenburg, 2022

Supervisor: Randa Akouri, M.D., Ph.D.

Cancer treatments often have gonadotoxic side effects leading to fertility dysfunction, especially in young cancer survivors. There are limited options for fertility preservation in prepubertal women undergoing gonadotoxic chemotherapy and radiation therapy. Although ovarian cortex transplantation (OCT) is the only method for these women to preserve fertility, there is concern that systemic cancer may metastasize to the ovaries, risking the reintroduction of tumor cells at the time of OCT. Therefore, these women need other alternative methods to maintain fertility that eliminate the risk of malignant cell re-implantation. The aim of this study was to develop an alternative fertility preservation method based on the ewe whole ovary ex-vivo perfusion platform and to investigate its feasibility. Eight ewes' ovaries were divided into two groups (groups 1 and 2) and perfused in bioreactors for up to 6 days. Group 1 (n=4) was stimulated with a single daily dose of human menopausal gonadotropin (hMG), while group 2 (n=4) was stimulated continuously for 24 hours. There were significant differences in follicle density and oocyte maturation in perfused ovaries in group 2 compared to the other group. Two groups collected a total of 22 oocytes. This study is the first to describe the recovery of MII oocytes after perfusion of whole ewe ovary.

Quantification of chemotherapy sensitivity in glioblastoma cells using the cell division assay

By: Alexandra Hellberg

Bachelor thesis in Biomedical Laboratory science, University of Gothenburg, 2022.
Preformed at advanced cell culture department 12, Sahlgrenska University Hospital.

Supervisor and assistant supervisor: Pegah Johansson, PhD. Yasaman Shamshirgaran, PhD.

Background: Glioblastoma multiforme (GBM) is the most common malignant brain tumor in adults. With its rapidly development, endothelial proliferation, and necrosis the mean of survival is estimated to 6-12 months. Treatment involves surgical removal of tumor followed by radiotherapy and chemotherapy. Temozolomide (TMZ) is the main drug used in chemotherapy although half of the patients develop resistant to the TMZ. Therefore, a method to optimize treatment for each tumor would help for personalized treatment (2). The aim of this study was to determine the feasibility of using cell division assay (CDA) to measure drug sensitivity in glioblastoma cells from different patients. We hope that by developing the assay we would be able to assist in the choice of treatment given to GBM patients.

Methods: Glioblastoma tumor cells were obtained from Assoc. Prof. Caren's group at Gothenburg University. Cells were treated in vitro with different cancer treatments including chemotherapy and radiotherapy and analyzed using the CDA. This involved incubation of the cells for two days after the treatment. To detect the dividing cells, thymidine analog 5-ethynyl-2′-deoxyuridine (EdU) is added for an additional day. the EdU positive cells were visualized according to a click-it protocol and quantified by flow cytometry. The EdU positive cells were then counted and cell sensitivity was calculated in treated samples relative to a non-treated sample from the same patient cells as reference for maximum proliferation.

Results: A distinct pattern of decrease in mean in CDA-Index was observed with given treatment and the results showed agreement between the CDA-Index and cell sensitivity. Variation between replicated experiments were also seen.

Conclusion: The study ultimately showed that the CDA could be used to measure sensitivity to DNA-damaging treatment in GBM tumor cells. Further optimization could improve the variation in the method. The assay indicates that the treatment should be adjusted for individual patients.

Testing of the glucose oxidase-DAB-nickel method for immunohistochemistry on kidney

By Clara Henningsson

Bachelor thesis in Biomedical Laboratory Science performed at Sahlgrenska Center For Cancer Research/Department of Clinical Pathology, Univerity of Gothenburg 2022.
Supervisior: Martin Johansson, Professor of Patologi

The kidneys are, on both functional and structural levels, very complex organs. It is of great reaserch interest to use histological and immunochemical methods to visualise the tissue. For histolpathological diagnosis of kidney diseases, histological examinations are absolutley crutial. But conventional immunohistochemical staining can often give non-specific staining of the tissue which can be missleading. In addition, the signal is often to heavy to allow a more fine-grained visualisation of the tissue. This Bachelor thesis aimed to introduce a histopathological protocol, for visualisation of thin structures, based on a chemistry with nickelenhancement and glucose oxidase. Instead of detection with conventional biotin-steptavidin technique a protocol based on the EnVision FLEX-system, with glucose oxidase-DAB-nickel instead of the DAB otherwise used in conventional staining with EnVision FLEX in Autostainer, is used. The glucose oxidase gives slower tissuestaining with greater detail, whilst nickel contributes by turning the colour of the DAB-product to black, instead of the otherwise brown product. The protocol was tested with antibodys used in histopathological diagnostics and in reasearch context with antibodys like LBR, HIPK3 and HMGA2.

The results from the nickelprotocol was compared to stainings done with Autostainer to determine differences in sensitivity, specificity and colurdifference. Further another nickelprotocol, based on Vectastain Elite-detection, is compared with the EnVision FLEX-based system. The results from the staining varied between different antibodys and dilutions. What all the stainings from the nickelprotocol had in common was that the positive celles was black and the stainign was more detailed and had less non-specific backgroundstaining compared to sections stained with the Autostainer. However, the antibodys could not be diluted as much with the nickelprotocol as with the Autostainer. The Vectastain-protocol resulted in more positive cells but the overall tussue also had a lot of unspecific staining. From this the concloution could be made that the nickel-protocol has worked and resulted in fine and detailed stainings, but would need to be developed a bit further, especially with longer inucbation time for the primary antibody, to achieve even better results.

MIC-values for species part of a model community for gastric flora established in Brain-Heart-Infusion broth

By Tora Hulterström

Bachelor thesis in Biomedical Laboratory Science performed at the Johan Begtsson-Palme lab at the department of infectious diseases, Sahlgrenska Academy, University of Gothenburg, 2022. Supervisors: Johan Bengtsson-Palme, associate professor, and Mirjam Dannborg, doctoral student.

Little is known about how antibiotics, even at sub-inhibitory concentrations, affects the gastric flora and the interactions between its species. Studying interactions of a complex flora *in vivo* is difficult. The use of a model community makes it possible to do it in controlled and reproducible settings. To investigate how sub-inhibitory concentrations affect the model community, minimum inhibitory concentrations first need to be established. In this project minimum inhibitory concentrations were established for the a few commonly used peroral antibiotics (cephalexine, clindamycin and ciprofloxacin) for a few of the community members (*Pseudomonas aeruginosa*, *Escherichia coli* and *Streptococcus salivarius*). The values were established with micro-broth dilution with Brain Heart Infusion broth and in both aerobic and microaerobic conditions. Minimum inhibitory values did not differ greatly between microaerobic and aerobic conditions. *Pseudomonas aeruginosa* and *Escherichia coli* showed, as expected, no or little inhibition by clindamycin even at the highest concentrations. *Pseudomonas aeruginosa* was not inhibited by cefalexin either, but was inhibited by ciprofloxacin at 2 mg/L (aerobic) and 4 mg/L (microaerobic). *Escherichia coli* was inhibited by cefalexin at 512 mg/L and by ciprofloxacin at 2 mg/L. *Streptococcus salivarius* was inhibited by cefalexin and ciprofloxacin at 2 mg/L and by clindamycin at 0.06 mg/L (aerobic) and 0.125 mg/L (microaerobic). The values are not comparable with those established with standard methods as both the broth and inoculum size used here differ from the standard. Instead these values reflect what the inhibitory concentrations might be in the finished settings for the model community. The final inhibitory concentrations will enable studies of interactions in sub-inhibitory concentrations. In the long run, this can contribute to greater understanding of how the use of peroral antibiotics affects gastric health.

Comparison between microscopic- and digital differential count from bone marrow

By Viktor Jartoft

Bachelor thesis in Biomedical Laboratory Science performed at the Bone marrow laboratory, Sahlgrenska University Hospital, Sahlgrenska Academy, University of Gothenburg, 2022
Supervisor: Sofia Grund, Consultant, Med. D.r.

Bakground: bloodmalignancy is diagnosed through the study of cells which reside in peripheral blood or the bone marrow. The examination is called morphological examination and uses a lightmicroscope, it's labour intensive and very time-consuming. Currently, an alternative method for analysis of peripheral blood smears (PBS) exists, which is digital and more efficient timewise, but not for bone marrow smears (BMS). A company called CellaVision® are developing a likewise instrument for BMS.

Purpose: the goal of this work is to, in a pilot study, train the neural network to recognize the morphological features of any leukocyte. To evaluate the quality of the chosen areas for differential count. To study the system's ability to classify. To compare the traditional differential counting method with the digitalized one.

Method: A total of Five BMS were used for differential counting. The digital method used low-resolution (10x) and high-resolution (100x) pictures to create areas for cell classification. Then the comparison, for each cell type, between the microscopic and digital assessment was done with the help of Equalis recommended limits for a 95 % confidence interval.

Results: Forty-four of the fifth-five comparisons were vaild according to their confidence intervals. The time-aspect for each method was estimated from the expert morphologist. The statistics from the pre-classification could not be evaluated.

Conclusion: The comparison between the microscopic- and digital method had many sources of error, but nonetheless the result from the comparison showed a relatively well conformity. The quality of used regions was deemed adequate for differential count. The question of the neural networks ability to classify cells is still unanswered, the same goes for the timesaving. The process of training the neural network to classify cells was done indirectly while working on to correct or agree with the systems pre-classification.

SUCCESSFUL DEVELOPMENT OF INDIRECT ELISA FOR COMMON COLD CORONAVIRUSES AND SARS-COV-2 USING THE SPIKE-PROTEIN AS ANTIGEN

By Sandra Jenneborg

Bachelor thesis in Biomedical Laboratory Science performed at the department of infectious diseases, institution of Biomedicine, Sahlgrenska Academy, University of Gothenburg, 2022.

Supervisors: Tomas Bergström, Professor, Linn Persson Berg, PhD student and Johan Ringlander, PhD student.

Background: The different endemic human coronaviruses (HCoVs) NL63, -OC43, -HKU1 and -229E cause upper respiratory infection. In 2020 a new coronavirus was detected, SARS-CoV-2, the cause of a global pandemic. The similarities between coronaviruses have led to the discussion if cross immunisations occur. Better methods to evaluate immunity after infection with HCoVs are necessary.

Aim: The aim of this study is to determine the optimal concentration of antigen to be used when developing specific ELISA methods for the detection of IgG antibodies to HCoV-NL63, -229E, -OC43, -HKU1 and SARS-CoV-2.

Method: The spike 1 protein (S1) of HCoV-NL63, -229E, -OC43, -HKU1 and SARS-CoV-2 was produced at the core facility Mammalian Protein Expression, Sahlgrenska Academy and used in indirect ELISA. Serum samples from patients that had been PCR-positive for the viruses and serum samples from 12-month-old children, were analysed to find positive and negative controls. These were then used to identify the optimal antigen concentration for indirect ELISA. The tested concentrations were 0.5, 1, 2 ,3, 4 and 5 µg/ml.

Result: Positive and negative control samples with adequate antibody responses were identified. There were two antigens for HCoV-NL63 e.g., NL63 S1 and NL63 S1 cleaved. The optimal concentration to use for both was 3 µg/ml, but NL63 S1 cleaved showed higher reactivity ($p<0.001$). For 229E S1, the highest reactivity was obtained with 5 µg/ml. SARS-CoV-2 produced in Human Embryonic Kidney (HEK) cells only showed reactivity with 3 µg/ml. OC43 S1 had similar reactivity between 2, 3 and 5 µg/ml. For HKU1 S1 and SARS-CoV-2 S1 produced in Chinese Hamster ovary (CHO) cells, all concentrations that were tested showed high and similar reactivity except 0.5 µg/ml.

Conclusion: The study demonstrated a successful development of sensitive, specific, and robust coronavirus S1 IgG ELISAs. The methods presented in this study may be used to investigate if cross-reactive antibodies to the various coronaviruses can be identified.

Cellular response to cardiac arrest

Potential activation of regenerative processes

By: Helen Jinton

Bachelor thesis in Biomedical Laboratory Science performed at the department of biomedicine, Sahlgrenska Academy, University of Gothenburg, 2022.
Supervisor: Kristina Vukušic, leg BMA, PhD.

Background: Cardiovascular diseases affect millions of people around the world and is a continuously growing group of diseases. The heart is known for its lack of regenerative capacity in response to injury. Injury to the heart more than often result in the formation of scar tissue, and for a long time cardiomyocytes were considered to be terminally differentiated cells. Recent studies however have shown that new cardiomyocytes can are produced, although to a low rate. The aim of this study was to investigate whether hypoxia induced by cardiac arrest, would activate regenerative processes in myocardium like for example de-differentiation or cell formation by stem cells.

Material and methods: The unique tissue material used for this study consisted of cardiac muscle biopsies from 5 healthy organ donors and 7 who had suffered a cardiac arrest. The biopsies consisted of the left ventricle and left atrioventricular junction, a region previously proposed as stem cell niche. With the use of immunohistochemistry, three different combinations of biomarkers were used to stain tissue sections from both locations. The biomarkers consisted of stem cell markers SSEA-4, MDR1, WT1, proliferation related Ki67, hypoxia marker HIF1α, and cardiomyocyte specific cTnT and PCM1. By the use of a fluorescence microscope tissue sections were photographed for analysis of the expression of biomarkers.

Results: The results consisted of a higher expression of stem cell and hypoxia related biomarkers in tissue sections belonging to donors who've had cardiac arrest. The differences between the atrioventricular junction amongst the two groups were not as high in comparison with differences in the left ventricle. An interesting result was the finding of potentially dividing cardiomyocytes with double PCM1+ nuclei, with a higher amount found in cardiac arrest tissue. Here a subpopulation of cardiomyocytes showed weaker cTnT expression and upregulation of MDR1 and SSEA-4.

Conclusion: The result of this study indicates that hypoxia has a role in activating regenerative processes in the adult heart. Whether this is by de-differentiation of cardiomyocytes or cell formation by stem cells is still unclear. Further research is required for a better understanding of the underlying mechanisms regarding regeneration of the human heart.

The stability of different analytes is comparable in BD Vacutainer® Barricor™ and BD Vacutainer® PST™

By Sandra Johansson

Bachelor thesis in Biomedical Laboratory Science performed at Laboratory Medicine, Clinical Chemistry, Falun Hospital, 2022.

Supervisor: Fredrik Bökman, Hospital Chemist, Ph.D.

There are speculations whether the newer plasma tube on the market BD Vacutainer® Barricor™ can improve the stability of analytes as well as other aspects of the analysis process. The purpose of this study is to compare if seven different analytes stability improves and hemolysis index becomes less with Barricor™ against PST™/ PST™II. Lactate dehydrogenase will also be compared with SST™II. Observe how the mechanical separator affects the probe at a small sample amount, how much sample amount is required for the probe to take plasma for analysis and compare how much plasma can be obtained from the tube at the recommended filling rate. This was done by blood sampling on 22 donors as well as 5 extras for analysis of plasma level. The tubes were centrifuged within 2 hours after sampling. The tubes were analyzed on instruments, the measurement values were converted into averages that were further used to calculate difference and statistical data. The results of this data are that aspartate transaminase, potassium and lactate dehydrogenase differ significantly in stability for Barricor™ from PST™. Lactate dehydrogenase is most stable for SST™II. The percentage of plasma differences between Barricor™ and PST™ differs only 2,74 percent. Hemolysis index measurement values are minimal but differ greatly for the coefficient of variation and significance. The conclusion drawn from these results is that the stability of Aspartate transaminase, potassium, and Lactate dehydrogenase increases in Barricor™ but not significantly for residual analytes. Lactate dehydrogenase stability was best in SST™II. Hemolysis index had small values which makes the results of these measurements a bit unreliable. Plasma required for analysis in Barricor™ was more than it was required for PST™ which could be a result of the curvature of the separator. The probe of the clinical chemistry instrument was not visibly affected by low plasma levels. Lastly the plasma levels in Barricor™ when compared against PST™II was only 2,74 percent more, which means that Barricor™ cannot be considered better than PST™II in this regard.

Optimization of preanalytical steps before analysis of cannabis in oral fluid

Stability of Δ9-tetrahydrocannabinol and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol in oral fluid sampled with the Saliva Collection Kit from Greiner Bio-One

By Lina Jönsson

Bachelor's thesis in Biomedical Laboratory Science performed at the Sahlgrenska University Hospital, University of Gothenburg, 2022.
Supervisors: Moa Andresen Bergström, PhD, Senior Analytical Chemist and Hanna Lövgren, Analytical Chemist

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Cannabis is the most frequently used illegal drug worldwide and therefore the most common substance that individuals are drug tested for. Use of oral fluid for drug testing has become more common because of its effective sampling, which is neither privacy-infringing nor invasive. When analyzing cannabis for substance abuse, Δ9-tetrahydrocannabinol and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol are interesting. However, these cannabinoids are lipophilic and tend to adsorb to lipophilic surfaces (such as plastics), which leads to analytical challenges as well as impaired stability and solubility in oral fluid samples. To improve the stability of these cannabinoids, previously published studies have used Triton X-100, a surfactant that is unfortunately planned to soon be banned in the EU. Therefore, this study aims to improve the stability of these cannabinoids by adding surfactant or solvent to oral fluid samples collected with kit from Greiner Bio-One to increase their solubility and reduce the adsorption to the polypropylene tube during the preanalytical phase. The project also aims to study how different storage conditions impacts on the stability of these cannabinoids. To fulfill these purposes, various experiments were carried out with more specific purposes in which spiked oral fluid samples were added with surfactant or solvent to the polypropylene tubes or during the sample preparation, depending on the intention of the experiment. The samples were then analyzed using a LC-HRMS instrument. The results showed a variation in stability of the cannabinoids between the different experiments and the additives, but in general Triton X-100 gave the best stability for both cannabinoids and no other additive reached comparable levels. When studying different storage conditions, the results indicate that storage at 4°C is preferable for the stability of the cannabinoids. In conclusion, due to varying results and lack of time, it is not possible to determine an additive that improve stability of the two cannabinoids sufficiently and is also reproducible enough to be introduced in routine clinical work. Finally, storage at 4°C is optimal for stability of these cannabinoids and substitutes for Triton X-100 that can improve stability just as well of both cannabinoids have not been successfully demonstrated during this project. Future studies are therefore encouraged to continue to investigate a way to optimize stability of these cannabinoids in oral fluid.

Characterization of a protein complex linked to autism

By Manal Kakhi Helaly

Bachelor thesis in biomedical laboratory science performed at the department of clinical chemistry lab in Sahlgrenska university hospital in Gothenburg 2022.

Supervisors: Fredrik Sterky, Associate professor and Angela Molinaro, Post-doc

Introduction: Synapses are connections that transmit signals between nerve cells to convey and process information in the brain. The shape and properties of synapses depend on their molecular structure, which is thought to be due to the interactions between cell adhesion molecules across the synapse cleft. Neurexins are examples of presynaptic cell adhesion molecules that can bind to several different postsynaptic ligands and interact with secretory synapse organizers. CA10 and CA11 are homologous glycoproteins that are mainly expressed in the brain. The exact function of CA10 is still unknown but it has been shown that CA10 forms a stable complex with neurexin as intermolecular disulfide bonds are formed between conserved cysteine residues in both proteins. CA10 has been shown to regulate affinities of neurexins to postsynaptic ligands by blocking a heparan sulfate (HS) modification of neurexin. Knockout mice were created for CA10 and CA11 to determine the function and relation to the neurexin-complex. However, the mutation in the CA10 gene does not have a complete loss of the CA10 protein, but about 20% of a ("hypomorphic") CA10 version remained

Aim: Characterization of the hypomorfic version of CA10 in vitro to determine if it functions the same way as the CA10 wildtype.

Method: We constructed a plasmid that expresses hypomorfic version of CA10 in HEK293 cells. This was done using PCR and Gibson assembly. We then transfected the HEK293 cells with different sets of plasmids which were then analyzed by immunoblotting, immunoprecipitation, surface-staining and microscopy.

Results: In immunoblots done in reduced and non-reduced conditions we observed that the hypomorfic version of CA10 could not form a proteincomplex with Nurexin nor CA11. By performing a surface-staining to investigate if a proteinkomplex would form on the cellsurface of Hek293-cells we found that no complexes were formed by the hypomorfic version of CA10 to Nurexin on the cellsurface, which supported our results from the immunoblots.

Conclusion: The hypomorphic version of CA10 is expressed at low levels in medium and it does not bind to Nrxn and is probably not functional, suggesting that the behavioral phenotype observed in mice is probably not due to the remaining 20% of truncated CA10 protein in the brain.

ANALYSIS OF P13α – A NEWLY DISCOVERED SPLICE VARIANT OF THE ENZYME PI3K

By: Sandra Klintstaf

Bachelor thesis in Biomedical Laboratory Science performed at the Clinical chemistry laboratory at Sahlgrenska Academy, University of Gothenburg, 2022

Supervisor Victoria Rotter Sopasakis, Ph.D, Associate Professor, Dept. Clinical Chemistry

Background: Type 2 diabetes is the most common form of diabetes. Insulin is required for glucose uptake, insulin activates intracellular signals, Phosphoinositide 3-kinase and Ras-mitogen-activated protein kinase signaling. Phosphoinositide 3-kinase is an enzyme that has a regulatory subunit, p85 and a catalytic subunit, p110. In the catalytic subunit there is p110α and it has been seen to be an important part in insulin signaling. p110α the most mutated gene in cancer, therefore there is a potential link between diabetes and cancer with respect to p110α. The research team has discovered a new splice-variant of p110α, p13α that encodes a smaller protein with only one intact domain, the p85-binding domain and does not have the catalytic domain, yet it induces cell proliferation in vitro and activates Akt. It has been found p13α in colon tumor tissue but not in healthy colon tissues, however, p13α was also found expressed 10cm from the tumor, which may indicate that p13α is an early tumor marker and involved in early tumor progression. Thus, the aim of this project is to elucidate the molecular mechanisms behind the p13α properties and its importance for metabolic and cell growth reprogramming

Method: Two different models were used, in vitro model (HEK239-cells) and in vivo model (Drosophila-fly). HEK293-cells were used for insulin signaling pathway via gene screening by Qiagen, the screening used qPCR with SYBR Green. For the Drosophila-fly, the genes of interest from the result of the HEK293-cells were proceeded, the method used was qPCR with Taqman probe, the flies had two different diets, high-sugar diet for diabetic condition and normal diet.

Results: The genes selected from the HEK293-cells generally did not show the same level of p13α-induced regulation in Drosophila. One gene that was significantly downregulated in Drosophila samples expressing p13α compared to control samples was ERCC1 by 80%.

Conclusion: In conclusion, the splice-variant p13α shows self-effect in vitro (HEK293-cells) that is distinct from the effects of the full-length protein p110α with respect to regulation of genes in the intracellular insulin signaling pathway. There was no difference in expression of genes in the diabetic flies, high-sugar diet compared to flies with normal diet, and this provided a difficult interpretation of the effect of p13α in combination with high-sugar diet.

Development of an effective enzymatic method for the analysis of, galactosylceramidase, regarding the investigation of Krabbe disease

By Sanna Lundh

Bachelor thesis in Biomedical Laboratory Science performed at the Department Of Clinical Chemistry, Unit Of Enzyme- and Metalanalysis, Sahlgrenska Academy, University Of Gothenburg, 2022.

Supervisor: Maria Blomqvist & Eva Johansson

Background: Krabbe disease is an inherited metabolic disease with absence or defiency of the lysosomal enzyme galactosylceramidase (GALC) which causes tissue damage such as demyelination and eventually early death. Symptoms such as spasm, rigidity, backward-bent body position and deteriorated motoric as well as cognitive functions occurs. The disease is inherited in an autosomal recessive pattern. In the current situation it does not exist a cure for Krabbe disease but early entry of HSCT treatment may delay the progression of the disease. Early diagnosis is significant to be able to offer prenatal diagnosis in upcoming pregnancies. Currently, a biochemical enzymatic method is used for diagnosis, utilizing the radioactive labelled substrate galactosylceramide. The analysis is time-consuming and the exposure of hazardous chemicals as well as labour-intensive methodology is a fact.

Aim: The purpose of this study was to improve the analysis of the enzyme GALC for diagnosis of Krabbe disease. By exchanging the radioactive substrate with a synthetic commercially produced fluorescent substrate the time of the analysis will be shortened, the exposure to hazardous chemicals will be reduced and existing labour-intensive methodology will be facilitated. This will lead to a more rapid biochemical diagnosis of Krabbe diease.

Methods: A fluorescent substrate, 6-Hexadecanoylamino-4-methylumbelliferyl b-D-galactopyranoside (6-HMU galactoside), was examined and the method was optimized. Individual blood and fibroblasts samples were investigated in this study to ascertain the specificity of the method and thereby distinguish between affected patient with Krabbe disease and control individuals. Leukocytes were isolated from 5 control individuals and 1 patient with Krabbe disease. Fibroblasts were cultured from 5 control individuals and 5 Krabbe patients. Isolation and lysis of cells, determination of the protein amount and the enzyme analysis were made to calculate the specific GALC-activity of the sample (µcatal/kg protein).

Results: The results showed that the 6-HMU galactoside substrate can be used with advantage to detect the enzyme GALC in leukocytes and fibroblasts. The method that was developed was able to differentiate between control individuals and Krabbe patients. Analytic steps in the existing method has been able to be completely removed or reduced such as the time of the analysis and the use of hazardous chemicals.

Conclusion: By introducing the fluorescent substrate, the time of the analysis was shortened, exposure of hazardous chemicals has been reduced and labour-intensive methodology is no longer a fact. However, analytical parameters have to be further optimized to improve assay specificity.

Evaluation of high-sensitive point of care analysis of cardiac-specific troponin I (cTnI)

An evaluation of Atellica VTLi from Siemens Healthineers

By Sofia Mohamed Osman

Bachelor thesis in Biomedical Laboratory Science preformed at the Department of Clinical Chemistry, Sahlgrenska University Hospital, 2022

Supervisor: Ola Hammarsten (Professor Senior Physician, Conjoint Professor)

Introduction: Cardiac-specific troponin is a protein complex specific to cardiac muscles and is essential for cardiac contraction. During ischemic injury and myocardial infarction, cardiac-specific troponin (cTn) is released from cardiomyocytes to plasma. Cardiac necrosis is therefore characterized by an increased concentration of cTn in plasma. Due to its specificity and its elevated levels in plasma after necrosis, cTn is now a central biomarker for diagnosis of myocardial infarction and determination of myocardial necrosis. The ability to quickly detect the biomarker in patients seeking emergency care for chest pain is prognostically important as this results in a more rapid implementation of treatment. Point of care analysis has the potential to quickly identify patients with ongoing myocardial infarction and implement treatment.

Aim: The purpose of the pilot study is to determine the performance of Atellica VTLi from Siemens Healthineers by performing a method comparison and precision study.

Material and method: Collection of cTnI samples was performed and frozen at -70 ° C. Frozen samples were thawed and centrifuged before analysis on Atellica. Method comparison on obtained cTnI values was performed between Atellica and Alinity by Abbott. The precision study was performed by analyzing samples once a day for 4 days.

Results: Regression analysis for method comparison shows that values from Alinity are higher than values from Atellica with a β0 of 3.15. With a β1 of 1.15 the result shows that values from Atellica increase by 1.15ng/L when values from Alinity increase by 1ng/L. The analysis also shows a R^2 of 33.1%. Bland-Altman plot speaks for the limits 95% of the difference is expected to appear and shows a range between 31.5 and -25.4 with a mean of 6.1. Coefficient of variation for between-day variation was calculated showing a variation for the samples between the intervals of 4.47%–14%.

Conclusion: In summary the relationship between cTnI values from the analytical instruments explain 33% of the variation between the values. High systematic error was also determined between the Atellica and Alinity. The pilot study also shows a high between-day variation, where 3 out of 5 samples had a CV >10%. The variation and systematic error are with high probability the result of samples being analyzed late, according to recommendations provided by Siemens Healthineers. For an increased understanding of the performance of Atellica future studies should be conducted including a larger sample size and newer sample material.

Titration of known irregular erythrocyte antibodies in pregnant women using IH-500

By: Dana Mohammad

Bachelor thesis in Biomedical Laboratory Science performed at Transfusion medicine, Södra Älvsborgs Sjukhus, University of Gothenburg, 2022

Supervisor: Mohammad R. Abedi, Maria Hermansson.

Immunization takes place when there is incompatibility between maternal antigen and fetal antigen. Antigen from the fetus leaks into the mother's blood via the placenta, and then the mother's immune system is activated and produces alloantibodies against the fetus' antigen. The specific IgG-type antibodies pass the placenta to the fetal bloodstream. The antibodies cause hemolysis of fetal erythrocytes, which is called Hemolytic Disease of the Fetus and Newborn (HDFN). The hemolysis of erythrocytes leads to fetal anemia and the release of fat-soluble unconjugated bilirubin which cannot be converted to conjugated bilirubin.

When hemolysis increases, bilirubin accumulates in tissues and crosses the blood-brain barrier, leading to kernicterus that results in permanent neurological damage. In a severe immunization reaction, a cascade of consequences occurs in the fetus which can lead to fetal death. It is important to titrate the alloantibodies during pregnancy to make a risk assessment if intrauterine blood transfusions or other postnatal treatment are needed.

The purpose of the study is to make a titer determination up to (1: 8) of irregular erythrocyte antibodies in pregnant women. The analysis titer determination was not available as an analysis at Transfusionsmedicin at Södra Älvsborgs Sjukhus (SÄS) in Borås, therefore there was a plan to validate and set up the analysis so the customer could get a faster response and Transfusionsmedicin SÄS could avoid sending samples to Gothenburg for titration at Sahlgrenska University Hospital.

A total of 43 samples were included in the study. The investigation showed some deviating results. Out of 43 samples, there were 18 samples which titration results did not match, which corresponds to 42%. The difference between the results from SÄS and the reference laboratory on the same sample was only one dilution step.

Titration results of irregular erythrocyte antibodies in pregnant women at Transfusionsmedicin SÄS have shown a good agreement with titration results from other reference laboratories. The analysis can be set up at SÄS instead of sending the samples to Sahlgrenska University Hospital.

Effect on amyloid precursor protein by dose-response inhibition of BACE in human iPSC derived neurons *in vitro*

By: Linnéa Mundin

Bachelor thesis in Biomedical Laboratory Science performed at the department of psychiatry and neuro chemistry, Institution of Neurology and Physiology, Sahlgrenska Academy, University of Gothenburg, 2022

Supervisor: Lotta Agholme, Researcher

Alzheimer's disease is the most common form of dementia. The hallmarks of Alzheimer's are extracellular beta-amyloid plaques and intracellular tau-tangles. Amyloid-beta (Aβ) is produced when amyloid precursor protein (APP) is proteolytically cleaved by the beta-secretase β-site APP-cleaving enzyme (BACE). Therefore, BACE inhibition has been a popular therapeutic target to reduce Aβ load with the aim to treat Alzheimer's disease. However, no BACE inhibitors have made it passed clinical trials, and several reasons for this have been suggested. First, we aimed to establish a dose-response curve for the BACE inhibitors Verubecestat and LY2886721 in human iPSC derived neurons. Thereafter, we wanted to investigate if dose-response BACE inhibition resulted in increased APP mRNA expression, intracellular accumulation, and altered cellular localization of APP. Human iPSC derived neurons were cultured for around 70 days. Cells were treated with the two BACE inhibitors: LY2886721 and verubecestat. The secreted Aβ38, -40, -42 was measured using electrochemiluminescence immunoassay. A dose-response curve was established using nonlinear-regression. After BACE inhibitor treatment, gene expression for APP, BACE1 and BACE2 was measured using qPCR. Protein levels of APP and BACE1 were measured using Western blot and using immunocytochemistry and confocal microscopy. The IC50 for verubecestat on human neurons was found to be 0,842 nM for Aβ40 and 0,776 for Aβ42. Verubecestat treatment did not alter APP levels or showed any dose-response effect. An upregulation of BACE2 was observed for all doses. We found that APP was not accumulating in the cells after BACE inhibition. Cells seem to respond to BACE1 inhibition by upregulating BACE2. The next step is to investigate eventual upregulation of ADAM10 and APP localization within the cells after BACE1 inhibition.

Quality control of blood components regarding leukocyte concentration, platelet yeild and the storage of erythrocytes.

By Julia Larsen Olofsson
Bachelor thesis in Biomedical Laboratory Science performed at the component laboratory, Sahlgrenska University Hospital, 2022
Supervisor: Camilla Hesse, senior lecturer

Background. Quality control is an essential part of establishments that handle blood components meant for transfusion. To ensure quality, tests of various parameters in the blood units are performed continuously. Among other things, leucocytes are reduced to <1/µL in the components to minimize the risk of transfusion-associated complications and the number of platelets in platelet concentrates is controlled. Another important part of quality work is the storage conditions of the components. For example, the erythrocyte units are stored in a Saline-Adenine-Glucose-Mannitol solution (SAGM) to maintain their quality during storage. **Aim.** The purpose of this study was to verify a new instrument, ADAM-rWBC (ADAM), for the control of leukocyte content in blood components and to increase knowledge about the quality of erythrocytes and platelets by extended quality controls.

Method. To verify a new instrument for the control of leukocyte content, 83 units (33 erythrocyte units, 25 plasma units, 25 platelet units) were analysed with both ADAM and Leukocount. For the monitoring of platelet yield during the making of pooled platelet concentrates, the platelet concentration was measured three times during the production of 10 pooled concentrates. In addition, several metabolic parameters were measured in erythrocyte concentrates over a 14-day period using a blood gas instrument.

Results. The results for the verification of ADAM show a statistically significant difference from Leukocount. However, the instrument is still considered acceptable for use as the differences are seen in the higher concentrations which rarely occur in routine quality controls. The analysis of platelet yield showed a 19% general decrease in platelet concentration from whole blood to the mini-platelet and a 33% general decrease from the mini-platelets to the pooled platelet concentrates. The large decrease is thought to be due to aggregates in the mini-platelets which cannot pass through the filter used for leukocyte reduction. For the analysis of erythrocyte units over time, a decrease in pH, glucose and sodium and an increase in lactate and potassium are seen. Potassium increased to 8 mmol/L after 3 days for two units, which is remarkable and should be investigated further. **Conclusion.** In summary, the study has shown that the instrument ADAM can be used for the control of leukocyte content in blood components and that platelet yield can be optimised. Moreover, the increased concentration of potassium needs to be investigated further.

Use of alternative software is possible for repeated strain measurements in breast cancer patients

By Sara Persson

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Physiology, Sahlgrenska Academy, University of Gothenburg, 2022

Supervisors: Anita Persson, PhD and Lovisa Doracic

Background: In 2020, breast cancer was the most common form of cancer in the world with 2.3 million new cases. In 15 – 30% of cases, a growth factor receptor, HER2, is expressed, which is associated with tumor growth, metastases and high mortality. With improved treatment, mortality has been reduced, but the risk of cardiotoxicity increases. Using speckle tracking echocardiography, the wall deformation in the left ventricle can be evaluated by measuring global longitudinal strain (GLS). Early subclinical changes, which are signs of cardiotoxicity, may be detected during repeated GLS measurements.

Aim: The aim of the study was to observe the agreement for GLS measurements in the left ventricle made with two different software (AFI and AutoStrain) from three consecutive examinations in breast cancer patients. Furthermore, the intention was to study performers´ measurement variation.

Method: The GLS value from breast cancer patients´ three previous echocardiographic examinations performed with AFI by various biomedical analysts was compared with the GLS value that the student received when measuring with AutoStrain. The study included 34 patients and of these, measurements were performed twice with AutoStrain on 15 patients.

Results: Measured GLS values with AutoStrain followed the same trend as previous measurements performed with AFI. There was no significant difference in measured GLS values between the software for each examination (GLS1: 20.1±1.9% respectively 20.3±1.6%, p=0.251, GLS2: 20.2±2.5% respectively 20.3±1.9%, p=0.736 and GLS3: 19.8±1.9% respectively 20.0±1.5%, p=0.394). The performer variation was within the clinic´s reference of 10% (GLS1: 5.0%, GLS2: 6.0% and GLS3: 4.7%). When one and the same performer made GLS measurements, a good agreement with low variation was seen.

Conclusion: The software AFI and AutoStrain showed good agreement and the measurements followed each other over time. The result demonstrates that both software can be used when the patient comes for repeated examinations.

Characterization of human fecal isolates from healthy individuals: anaerobic culturing and molecular methods for identification

By Maria Rutgersson

Bachelor thesis in Biomedical Laboratory Science performed at the Wallenberg Laboratory, Sahlgrenska Academy, University of Gothenburg, 2022

Supervisors: Valentina Tremaroli, Researcher
Johanna Jönsson, Biomedical Scientist

Background: The human gut microbiota plays an important role in human health and disease. Metagenomics and improvements of anaerobic culturing techniques have increased our knowledge on the microorganisms, and particularly Bacteria, that live in the gut, although recent research has shown that a relevant portion of the bacterial species found by metagenomic sequencing still lack cultured representatives. Therefore, the aim of the project was to characterize bacterial isolates obtained from stools of one healthy human donor with anaerobic culturing on a range of growth media, to possibly identify previously uncultured gut bacteria of human origin.

Material and Methods: 20 isolates were tested for purity, subjected to Gram staining and identified by sequencing of the 16S rRNA gene. 9 of the 20 isolates were also subjected to API 20 A system.

Results: The results indicated the successful isolation of several uncultured organisms in addition to *Escherichia coli* and *Clostridium* species.

Conclusions: Gram staining and sequencing of the 16 S rRNA gene are powerful tools to characterize unknown isolates. In this project the analysis of the 16S rRNA gene sequences obtained for 16 of the isolates indicated that the sequence of 5 isolates was highly similar to uncultured bacteria whose 16S rRNA gene sequences are present in the database but so far lack characterization. The methods used in this project were not sufficient for their characterization, and therefore genome sequencing might be required in the future.

ABSTRACT

GENETIC DELETION OF *BPP43_05035* IN BRACHYSPIRA PILOSICOLI

By Fadumo Mohamed Salad

Bachelor thesis in Biomedical Laboratory Science performed at Department of medical biochemistry and cell biology, Institute of biomedicine, University of Gothenburg, 2022
Supervisor: Thaher Pelaseyed, PhD, Assistant Professor (Forskarassistent)

Introduction: *Brachyspira pilosicoli* is an anaerobic spirochete that permeates the mucosal layers of the large intestine and colonizes epithelial cells causing Intestinal spirochetosis. The gene *BPP43_05035* is speculated to have an important function in *B. pilosicoli* adherence to intestinal epithelial cells.

Aim: The aim of this research project was to delete *BPP43_05035* in the *B. pilosicoli* by replacing it with an ampicillin resistant gene using homologous recombination.

Method: A deletion cassette was constructed by using the vector pcDNA3.1 and *B. pilosicoli* genomic DNA as templates and each fragment were amplified using PCR and verified by separation on agarose gel. Gibson assembly was used to assemble the fragments. Electrocompetent cells were prepared and electrotransformed with pGFPuv.

Results and conclusion: Sequencing analysis of the deletion cassette showed that it was constructed, and that sequence was correct and free from mutations.. Electrotransformation of *B. pilosicoli* with the plasmid pGFPuv showed that protocols need to be optimized for successful transformation of *B. pilosicoli*.

The NAD^+-modulator KL1333 improves pyruvate concentration in rotenone-inhibited human leucocytes

By: Emelie Salamon

Bachelor thesis in Biomedical Laboratory Science performed at Mitochondrial Medicine, Department of Clinical Sciences, Lund University, 2022.
Supervisors: Eskil Elmér, adjunct professor, Imen Chamkha, postdoc.

The mitochondrion is one of the cells most important organelles, mainly due to the important role in energy production. Mitochondrial disorders can occur as a result of mutations in either the nuclear or mitochondrial genome. This is a diverse disease group that varies greatly in severity and clinical presentation. There is no current cure for mitochondrial diseases and treatment strategies primarily focus on reducing symptoms. Therefore, there is a great demand to develop new pharmaceuticals that targets the disease mechanism in patients with impaired mitochondrial function.

The main purpose of this study was to investigate the effect of the NAD^+-modulator KL1333, by analyzing extracellular levels of lactate and pyruvate, in an *in vitro* model of mitochondrial disease.

Leukocytes from healthy blood donors were isolated and inhibited by rotenone, a complex I specific toxin. Leukocytes with simulated complex I dysfunction were then treated with the drug candidate KL1333. Extracellular concentrations of lactate and pyruvate were analyzed every 30 minutes for two hours with the ISCUSflex Microdialysis Analyzer. Complex I-inhibited cells with treatment were compared with inhibited cells without treatment, and as well as with control cells without inhibition or treatment.

Rotenone significantly decreased the release of pyruvate from human leukocytes. The pyruvate concentration increased significantly ($p = 0.0078$) in complex I inhibited leukocytes treated with KL1333 ($m = 26.00$ μM and 95% CI = 19.02 - 32.98 μM), compared to inhibited cells without treatment ($m = 10.38$ μM and 95% CI = 5.91 - 14.84 μM). However, no significant difference in lactate concentration was detected between the different groups.

In conclusion, the study showed that KL1333 restores the extracellular levels of pyruvate in human leukocytes with simulated complex I dysfunction. This strengthens the hypothesis that KL1333 can act as a future treatment for mitochondrial diseases.

Metal artifact reduction on CT improves PET attenuation correction

By: Simone Segerfelt

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Physiology, Sahlgrenska Academy, University of Gothenburg, 2022
Supervisors: Jakob Himmelman, Msc and Martijn Van Essen, MD, PhD

Introduction: Positron emission tomography (PET) in combination with computed tomography (CT) is a valuable method in tumor diagnostics. Although PET/CT is well-established, some limitations remain regarding metal implants that cause artifacts in the CT image. The problem is particularly significant in PET/CT because PET attenuation correction relies on CT data. To reduce CT artifacts, progress have been made with metal artifact reduction (MAR) algorithms; Smart-MAR and iterative MAR. However, there have been insufficient research if metal artifact reduction algorithms affect PET attenuation correction. Within Sahlgrenska University Hospital, a standard method for PET attenuation correction is used instead, which does not reduce metal artifacts. Hence, this study aimed to investigate if iterative MAR and Smart-MAR affect attenuation correction and image quality in PET.
Method: To investigate the impact of iterative MAR and Smart-MAR a phantom study was conducted, followed by clinical PET/CT images of 27 patients that were analyzed. The effects of iterative MAR and Smart-MAR were analyzed in areas with and without metal artifacts. Standardized uptake value was used for quantification of tracer uptake. The results from the phantom study were used to verify the result from the clinical PET/CT images. Finally, reconstructed PET images were studied visually.
Results: A statistically significant difference was found for standardized uptake values obtained in areas with artifacts between reconstructed PET images, when using the standard method compared to iterative MAR. No difference was found in areas without artifacts. In the visual assessment, no clear visual difference could be observed. Smart-MAR in CT image for PET attenuation correction resulted in lower quantification compared to the standard method.
Conclusion: CT reconstruction with iterative MAR for PET attenuation correction resulted in more accurate quantification. In addition, iterative MAR does not affect quantification in reference areas. Regarding Smart-MAR, further studies are needed before conclusions can be determined.

Method comparison between LC-MS/MS and Cobas 8000 for measurments of 25-hydroxyvitamin D in serum

By Eliza Simon

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Clinical Chemistry, Halland Hospital, Halmstad, 2022

Supervisor: Jan Miller, MD. Secondary supervisor: Per Bengtson Hospital Chemist, PhD

Introduction: Vitamin D is needed primarily for regulating calcium and phosphorus metabolism. Deficiency of this vitamin leads to development of rickets in children and osteomalacia in adults and is also associated with several other diseases such as certain cancers, autoimmune diseases, various infections, and cardiovascular diseases. Several methods to analyze vitamin D concentration and thereby detect vitamin D deficiency or vitamin D insufficiency exist. Halland routinely uses a liquid chromatographic method on LC-MS/MS, which is also the reference method in this study and is compared with the latest automated immunochemical method from Roche Diagnostics, Elecsys Vitamin D total III on the instrument Cobas 8000.
Aim: The aim of this study was to investigate the performance of the Elecsys Vitamin D Total III method on the instrument Cobas 8000, in the analysis of 25-hydroxyvitamin D concentration in serum. The method was compared with the manufacture's description, Halland Hospital's own requirements and existing routines used with the LC-MS/MS method.
Method: The total vitamin D concentration, S-25(OH)D was analyzed on 90 randomly selected serum samples on the LC-MS/MS analysis instrument and then on the Cobas 8000 analysis instrument. A precision study was performed on Cobas 8000 where mean, SD and CV was calculated for intraday variation after ten measurements per sample on three different patient samples with different concentration levels (low, medium, high) during the same day. Between day variation was measured on three different patient samples in different concentration levels (low, medium, high) performed during three consecutive days in triplicate. A correlation analysis was also performed to examine the relationship between the two methods, as well as a Bland-Altman diagram and a Passing-Bablok regression analysis to compare how well the methods agree with each other.
Results: The intraday variation for the low level (17.6 nmol / L) had a CV of 7.9%, the intermediate level (76.5 nmol / L) had a CV of 2.2% and the high level (126.8 nmol / L) had a CV of 2.3%. The between day variation for the low level (27.9 nmol / L) had a CV of 7.2%, the intermediate level (75.9 nmol / L) had a CV of 5.2% and the high level (117.6 nmol / L) had a CV of 2.7%. The determination coefficient was 93.98% and the regression analysis showed that there is no significant difference in the results between the methods.
Conclusion: The results obtained indicate that the methods have a good precision and consistency with each other and would support a decision to move S-25(OH)D analysis from LC-MS/MS to Cobas 8000. In addition to satisfactory test performance and shorter turnaround times, the new method would simplify the analysis of vitamin D and save time for laboratory staff.

Finding Neo: developing imaging methods for visualising *Neoehrlichia mikurensis* in blood

By Sofi Sjöberg

Bachelor thesis in Biomedical Laboratory Science performed at the Department of Infectious Diseases, Sahlgrenska Academy, University of Gothenburg, 2022.

Supervisors: Linda Wass, specialised biomedical scientist and PhD student, and Anna Grankvist, PhD.

Neoehrlichia mikurensis is believed to be the third most common tick-borne human pathogen, behind Borrelia and Rickettsia. It is an intracellular bacterium that causes neoehrlichiosis, a disease characterised by prolonged fevers, pain, and vascular events. As it does not grow on cell-free culture media, it cannot be diagnosed with routine microbiological methods. Instead, a *N. mikurensis*-specific real-time PCR is utilised.

The aim of this thesis project is to visualise *N. mikurensis* in plasma and buffy coat from patients with positive PCR-results, using Fluorescence in Situ Hybridisation (FISH) and immunocytochemistry (ICC). For this, a *N. mikurensis*-specific probe was used, along with an immune serum from an immunocompetent patient, and a rabbit antibody against an outer membrane protein unique to this bacterium. Different materials, slide preparations, fixatives, blocking-, permeabilisation-, and hybridisation buffers were tested, and the slides were imaged using confocal microscopy.

Acetone- or formal saline fixed buffy coat smears were determined to be the superior fixatives, material, and slide preparation. For ICC, it was found that the slides blocked with animal serum gave the least amount of background staining, and that the patient serum should be avoided in favour of the rabbit antibody. For FISH, the best results were achieved using TBS with 0,1% saponin as a permeabilisation buffer followed by PBS as a hybridisation buffer. Unfortunately, as both ICC and FISH resulted in non-specific staining, this study did not quite achieve its aim of conclusively visualising *N. mikurensis*. However, it did produce some important results that will be instrumental in the continuation of this project.

Blood volumes estimated in BacT/ALERT VIRTUO™ do not achieve new quality indicators

– Low blood volumes in blood culture vials make it difficult to detect sepsis

By Linn Stenson

Bachelor thesis in Biomedical Laboratory Science performed at the department Clinical Microbiology laboratory, Södra Älvsborg Hospital, Borås, 2022.
Supervisor: Claes Henning, docent, chief physician.

Introduction: Blood culture can detect which pathogen causes sepsis and, if necessary, correct a primary antibiotic treatment. The amount of bacteria (CFU/mL) is low in the blood, therefore a large volume of blood is required. According to new quality indicators, the blood volume should be ≥8 mL per bottle in 95% of all blood culture bottles or ≥35 mL with 95% in a set of four blood culture bottles.

Aim: The purpose was to measure blood volumes in blood culture vials submitted for culture from adult patients with suspected sepsis, to investigate whether the volumes achieve the quality based on new recommendations for blood culture. The purpose also included investigating the reason why the automatic volume estimation does not always work.

Methods and materials: A validation was performed by Virtuo. Blood volume was calculated using weighing and automatic estimation of Virtuo. A retrospective survey was conducted from November to December 2021. Troubleshooting for low volumes was performed.

Results: 52% of the blood culture vials were filled with ≥8 mL and 45% of the blood culture vials were filled with ≥ 35 mL in a set of four vials. In addition, 25% did not receive any volume, of which 76% were anaerobic and 97% were scanned in Virtuo A1/B1. Misplacement of labels took place from 17.46 to 06.56. From retrospective examination, 49% were filled with ≥8 mL and 23% received no volume.

Conclusion: New quality indicators are not achieved, and low blood volumes are obtained during blood filling. Most bottles without volume are due to incorrect placement of a label that covers the column for volume estimation or QR code, which usually happens during the night shift.

The role of IL-6 in the ventral hippocampus: focus on depression and anxiety like behavior in rats

Matteus Svensson

University of Gothenburg, Göteborg, Sweden; Institute of neuroscience and physiology.

Purpose: The purpose of this study is to investigate the behavioral effects of a viral knockdown of interleukin-6 (IL-6) in the ventral hippocampus (vHPC) of male and female rats.

Theory: IL-6 is a pro-inflammatory cytokine known for regulating inflammatory responses. Evidence from recent studies show that IL-6 is a critical player in multiple psychological central nervous system (CNS) processes and may also contribute to the etiology of neuropathological disorders.

Method: Male and female rats were injected in the vHPC brain region with AAV-siRNA-IL6 to knockdown the expression of IL-6. To investigate the depression-like behavior of rats forced swimming test was utilized. *In situ* hybridization assay (RNAscope) was implemented to co-localize the expression of IL-6 in the vHPC in certain cell populations and with confocal imaging verify the reduced expression and determine the cellular origin. Real-time PCR were performed for detection of IL-6 receptor (IL-6R) and brain-derived neurotrophic factor (BDNF) in the vHPC.

Result: Microinjections of AAV-siRNA-IL6 into the vHPC region successfully and significantly reduced IL-6 expression by ~50%. Confocal images presented that IL-6 mRNA in the vHPC co-localizes with neurons (~53%), Astrocytes (31%) and microglia (16%). Knockdown of IL-6 expression in the vHPC reduced depression-like behavior in male rats. In contrast, female rats showed no significant difference in behavior. In addition, no significant difference was found with real time PCR detection of IL-6R or BDNF expression. Taken together, these results indicate that IL-6 in the vHPC play a significant role in the expression of depressive-like behavior in a sex specific manner.

A reduced count rate to 10-15 on the Radhound detector corresponds to the recommendation from EANM:s guidelines when preforming V/P SPECT

By Sohir Turki

Bachelor thesis in Biomedical Laboratory Science performed at the nuclear medicine department of Sahlgrenska University Hospital, Sahlgrenska Academy, University of Gothenburg, 2022.
Supervisor: Johanna Dalmo, phD and Jenny Ornestedt

Background: Pulmonary embolism is a situation in which a blood clot occludes a pulmonary artery, obstructing perfusion. The ventilation/perfusion single-photon emission computed tomography approach is utilized to produce an effective and reliable diagnosis through tomographic images. Ventilation is studied by allowing the patient to inhale a radioactive gas and an intravenous radioactive injection is administered to investigate perfusion. Two images are acquired and compared by constructing a quotient image, detecting pulmonary embolism where ventilation is normal, but perfusion is reduced.
Purpose: The main aim was to evaluate whether the perfusion activity needs to be adjusted depending on how the patient performed at the ventilation section, to achieve an ideal image ratio of 4 ± 1 as recommended by the European Association of Nuclear Medicine.
Method: The method consisted of 3 different steps. The collected data was first analyzed retrospectively to assess existing quotients at Sahlgrenska. Itt was then discovered that the ventilation activity was higher than expected. The project's aim was adjusted, and the ventilation activity was investigated further through a phantom study. The count rate from the gamma detectors were also evaluated on 10 patients chosen at random from the total number of participants. Patients whose images were not planar were excluded from the research.
Result: No weight-related influence on image quality was seen. The activity was determined to surpass EANM's guidelines based on the phantom study. The V/P quotient image at Sahlgrenska is within an approved limit since the count rate for ventilation is greater than predicted and the quantity of activity in the perfusion dose is in the upper half of the range authorized in EANM guidelines.
Conclusion: Sahlgrenska is advised to adjust the Radhound counting rate and invest in achieving a count rate of 10-15. In this way, the patient's perfusion dosage may not be subjected to unreasonably high levels of activity.

OPTIMIZATION OF A MULTIPLEX QPCR ASSAY FOR DETECTION OF SHIGATOXIN, INTIMIN AND O157:H7 IN EHEC

By Sofia Törnblad

Bachelor thesis in Biomedical Laboratory Science, Sahlgrenska Academy, University of Gothenburg, 2022. Performed at the Department of Clinical Microbiology, Halland.
Supervisor: Christoffer Lindsten

Escherichia coli occurs naturally in the microbiota of many vertebrates where it is apathogenic. There are, however, several pathotypes of *Escherichia coli* that can cause enteritis. One of these is the primary pathogen Enterohemorrhagic *Escherichia coli* which can colonize the human gut and cause diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome. The disease is transmitted by the fecal-oral route and is spread through contact with animals, contaminated food, or contaminated water. Enterohemorrhagic *Escherichia coli* has caused several large-scale outbreaks globally.

The Shiga toxins stx1 and stx2 as well as the adhesion protein intimin are important virulence factors. There are several different subtypes of stx1 and stx2. The gene that encode the Shiga toxins are called *stx* and for intimin *eae*.

Shiga toxin-producing *Escherichia coli* manifests itself in various O:H serotypes but is mainly associated with O157:H7. The O antigen is synthesized by the locus *rfb* whereas the H antigen is expressed by *fliC*.

The purpose of this study was to develop a protocol to be able to simultaneously detect or exclude the presence of i) stx1a, c, d and stx2a-d, f, g, l, m and ii) rfb_{O157} (encodes O-antigen for O157) and $fliC_{h7}$ (encodes H-antigen for H7) and *eae* in a multiplex real-time PCR.

An optimal concentration of oligonucleotide mixes containing target sequence-specific primers and probes for stx, O157, H7, and eae respectively was obtained by studying the amplification curves with a reference strain given by analysis in multiplex real-time PCR. Thereafter, the selected oligonucleotide mixes were run against 31 known clinical isolates in a real-time PCR to validate the assay. This study was able to detect all the stx variants mentioned above, able to identify O157:H7 with 100% agreement, and also able to find the presence of *eae*. Therefore, it is concluded that this study was relevant to the clinical practice.

Time Lapse Analysis Shows That New Sperm Injection Method Provide As Good Embryo Development As Conventional Method

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Background: Infertility is a global health issue and in reproductive medicine various assisted reproductive techniques (ART) are performed to treat this. One of these is intracytoplasmic sperm injection (ICSI) which is mainly used in the indication of male factor. A newer variant of this method, here called “direct ICSI”, has been discussed for its potential of an increased fertilization rate relatively the conventional method. The difference between the methods is how the injection techniques are used to produce holes in the oocytes elastic cell membrane.

Aim: The aim of this study is to compare direct ICSI with conventional method, with a time lapse analysis, to see if there is any difference after fertilization in cell division patterns and other morphokinetic parameters. A further goal is to investigate in a later randomized study whether there is a possibility to improve the current fertility rate using ICSI.

Method: 468 oocytes, of which 314 were fertilized and cultured to embryos, were obtained from 44 patients/couples who underwent treatment at Reproductive Medicine, Sahlgrenska University Hospital. The embryos were grown in a time lapse incubator (EmbryoScope+®, Vitrolife©) for a maximum of six days and monitored with time lapse. Supplementary annotations were made on pre-recorded material with timestamps (hours in tenths) for time intervals from fertilization to hatched blastocyst, as well as morphological objective parameters.

Results: The variable “embryo utilization rate” achieved a significant difference between the groups ($p = 0.029$), where a larger proportion of embryos in the direct ICSI group went to freezer and transfer. No difference for the other studied variables could be demonstrated.

Conclusion: This pilot study is the first to compare direct- and conventional ICSI using time lapse parameters. The results obtained concluded that there was no difference between the methods beyond the variable "embryo utilization rate". The conclusion from this study is that the new method is equivalent to the conventional method. Additionally, more and larger studies are needed to confirm these findings.